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# Lxr $\alpha$ Deficiency Hampers the Hepatic Adaptive Response to Fasting in Mice<sup>\*S</sup>

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Besides its well established role in control of cellular cholesterol homeostasis, the liver X receptor (LXR) has been implicated in the regulation of hepatic gluconeogenesis. We investigated the role of the major hepatic LXR isoform in hepatic glucose metabolism during the feeding-to-fasting transition *in vivo*. In addition, we explored hepatic glucose sensing by LXR during carbohydrate refeeding. *Lxr $\alpha$ <sup>-/-</sup>* mice and their wild-type littermates were subjected to a fasting-refeeding protocol and hepatic carbohydrate fluxes as well as whole body insulin sensitivity were determined *in vivo* by stable isotope procedures. *Lxr $\alpha$ <sup>-/-</sup>* mice showed an impaired response to fasting in terms of hepatic glycogen depletion and triglyceride accumulation. Hepatic glucose 6-phosphate turnover was reduced in 9-h fasted *Lxr $\alpha$ <sup>-/-</sup>* mice as compared with controls. Although hepatic gluconeogenic gene expression was increased in 9-h fasted *Lxr $\alpha$ <sup>-/-</sup>* mice compared with wild-type controls, the actual gluconeogenic flux was not affected by *Lxr $\alpha$*  deficiency. Hepatic and peripheral insulin sensitivity were similar in *Lxr $\alpha$ <sup>-/-</sup>* and wild-type mice. Compared with wild-type controls, the induction of hepatic lipogenic gene expression was blunted in carbohydrate-refed *Lxr $\alpha$ <sup>-/-</sup>* mice, which was associated with lower plasma triglyceride concentrations. Yet, expression of “classic” LXR target genes *Abca1*, *Abcg5*, and *Abcg8* was not affected by *Lxr $\alpha$*  deficiency in carbohydrate-refed mice. In summary, these studies identify LXR $\alpha$  as a physiologically relevant mediator of the hepatic response to fasting. However, the data do not support a role for LXR in hepatic glucose sensing.

Liver X receptors  $\alpha$  and  $\beta$  (LXR $\alpha/\beta$ ,<sup>2</sup> NR1H3/NR1H2) are important players in the transcriptional control of various met-

abolic pathways. LXR $\alpha$  is predominantly expressed in liver, intestine, and adipose tissue but is also present in kidney, lung, and spleen. LXR $\beta$  is expressed in almost all tissues and organs (1, 2). LXRs can be activated by oxidized cholesterol metabolites (oxysterols), which have been identified to be their natural ligands. Hence, LXRs act as intracellular “cholesterol sensors” (3). LXRs induce lipogenic gene expression upon activation, both directly (4) and indirectly via the transcription factors sterol regulatory element binding protein-1c (SREBP-1c) and carbohydrate response element binding protein (ChREBP) (4–7). Both SREBP-1c and ChREBP are involved in control of the conversion of glucose into fatty acids. Thus, LXRs coordinate the interactions between sterol and fatty acid metabolism, for instance to enable cholesterol ester formation during cellular cholesterol overload. In the past years, several studies have been published that point toward a role of LXRs in the control of glucose homeostasis. These studies showed that pharmacological LXR activation improves glycemic control in diabetic rodent models by increasing peripheral glucose disposal (8, 9) and/or inhibition of hepatic gluconeogenesis (9–12). Mitro *et al.* (13) recently reported that physiologically relevant concentrations of either glucose or glucose 6-phosphate (G6P) are able to bind and activate LXR in HepG2 cells. The physiological relevance of this potential “glucose sensing” role of LXR has been debated (14–16) and needs to be established.

To explore the physiological relevance of LXR in hepatic glucose metabolism we subjected mice deficient for *Lxr $\alpha$* , the major hepatic isoform, to a fasting-refeeding protocol. *Lxr $\alpha$ <sup>-/-</sup>* mice showed an impaired hepatic fasting response in terms of glycogen depletion and triglyceride (TG) accumulation. Although gluconeogenic gene expression was increased in 9-h fasted *Lxr $\alpha$ <sup>-/-</sup>* mice compared with wild-type mice, stable isotope infusion revealed the actual gluconeogenic flux was not affected by *Lxr $\alpha$*  deficiency. G6P turnover was reduced in *Lxr $\alpha$ <sup>-/-</sup>* mice compared with wild-type mice. In carbohydrate-refed *Lxr $\alpha$ <sup>-/-</sup>* mice, the hepatic lipogenic response was blunted while changes in the expression of the LXR target genes *Abca1*, *Abcg5*, and *Abcg8* were similar in wild-type and *Lxr $\alpha$ <sup>-/-</sup>* mice. Taken together, these data imply an important role for LXR $\alpha$  in the control of hepatic glucose metabolism upon fasting, but they do not support the hypothesis that *Lxr $\alpha$*  acts as a hepatic glucose sensor.

## EXPERIMENTAL PROCEDURES

**Animals and Diets**—F2 male *Lxr $\alpha$ <sup>-/-</sup>* mice and their wild-type littermates on a Sv129/OlaHsd C57Bl/6J mixed back-

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<sup>S</sup> The on-line version of this article (available at <http://www.jbc.org>) contains supplemental Fig. S1 and Table S1.

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<sup>2</sup> The abbreviations used are: LXR, liver X receptor; Abc, ATP binding cassette; Acc, acetyl-CoA carboxylase; ChREBP, carbohydrate response element-binding protein; Fas, fatty acid synthase; Fbp1, fructose-1,6-bisphosphatase 1; G6P, glucose 6-phosphate; G6ph, glucose-6-phosphate hydrolase; G6pt, glucose-6-phosphate translocase; Pgc-1 $\alpha$ , peroxisome proliferator-activated receptor  $\gamma$  co-activator 1 $\alpha$ ; Pepck, phosphoenolpyruvate carboxykinase; Pdk4, pyruvate dehydrogenase kinase 4; Scd1, stearoyl-CoA desaturase 1; SREBP-1c, sterol regulatory element-binding protein 1c; TG, triglyceride; NEFA, non-esterified fatty acid;  $\beta$ -HB,  $\beta$ -hydroxybutyrate.

# Lxra Mediates the Hepatic Response to Fasting

**TABLE 1**

**Plasma and liver parameters in *Lxra*<sup>-/-</sup> mice and their wild-type littermates**

Mice were fasted for 0, 9, or 24 hours. Blood glucose concentrations were measured using a EuroFlash glucose meter. Plasma insulin, NEFA,  $\beta$ -HB, TG, and cholesterol concentrations were determined using commercially available kits. Values represent means  $\pm$  S.E. for  $n = 4 - 6$ .

	Fed		9-h fasted		24-h fasted	
	Wild-type	<i>Lxra</i> <sup>-/-</sup>	Wild-type	<i>Lxra</i> <sup>-/-</sup>	Wild-type	<i>Lxra</i> <sup>-/-</sup>
Blood glucose (mM)	8.8 $\pm$ 0.3	9.0 $\pm$ 0.7	5.2 $\pm$ 0.3 <sup>a</sup>	4.8 $\pm$ 0.8 <sup>a</sup>	3.5 $\pm$ 0.4 <sup>b</sup>	3.6 $\pm$ 0.5
Plasma insulin (ng/ml)	1.59 $\pm$ 0.38	1.37 $\pm$ 0.58	0.12 $\pm$ 0.03 <sup>a</sup>	0.13 $\pm$ 0.1	0.06 $\pm$ 0.01	0.06 $\pm$ 0.02
Plasma NEFA (mM)	0.38 $\pm$ 0.04	0.48 $\pm$ 0.05	0.78 $\pm$ 0.05 <sup>a</sup>	0.68 $\pm$ 0.04	0.84 $\pm$ 0.03	0.78 $\pm$ 0.04
Plasma $\beta$ -HB (mM)	0.18 $\pm$ 0.04	0.23 $\pm$ 0.09	1.57 $\pm$ 0.31 <sup>a</sup>	1.12 $\pm$ 0.37	3.33 $\pm$ 0.25 <sup>b</sup>	3.72 $\pm$ 0.19 <sup>b</sup>
Plasma TG (mM)	0.46 $\pm$ 0.09	0.47 $\pm$ 0.10	0.79 $\pm$ 0.09	1.01 $\pm$ 0.25	1.25 $\pm$ 0.09 <sup>b</sup>	0.91 $\pm$ 0.17
Plasma cholesterol (mM)	1.8 $\pm$ 0.1	1.6 $\pm$ 0.1	2.4 $\pm$ 0.1 <sup>a</sup>	1.9 $\pm$ 0.1 <sup>c</sup>	1.8 $\pm$ 0.2	2.4 $\pm$ 0.4

<sup>a</sup>  $p < 0.05$  9-h fasted versus fed.

<sup>b</sup>  $p < 0.05$  24-h fasted versus 9-h fasted.

<sup>c</sup>  $p < 0.05$  *Lxra*<sup>-/-</sup> versus wild-type (Mann-Whitney  $U$  test,  $p$  value adjusted for multiple comparisons).

ground (17) were housed in a light- and temperature-controlled facility (lights on 7 a.m. to 7 p.m., 21 °C). They were fed standard laboratory chow *ad libitum* (RMH-B, Abdiets, Woerden, The Netherlands) and had free access to water. All experiments were approved by the Ethics Committee for Animal Experiments of the University of Groningen.

**Fasting and Refeeding Experiments**—For fasting experiments we studied separate groups of mice. All mice were killed by cardiac puncture under isoflurane anesthesia at 8 a.m., either without being fasted, after a 9-h fast, or after a 24-h fast. For the refeeding experiments, mice were killed at 8 a.m. after a 24-h refeeding period with free access to high carbohydrate chow (38.5% w/w sucrose, Abdiets) following a 24-h fasting period.

**Plasma Metabolite Concentrations**—Blood glucose concentrations were measured using a EuroFlash glucose meter (Lifescan Benelux, Beerse, Belgium). Plasma insulin concentrations were determined using enzyme-linked immunosorbent assay (Ultrasensitive Mouse Insulin kit, Mercodia, Uppsala, Sweden). Plasma non-esterified fatty acid (NEFA),  $\beta$ -hydroxybutyrate ( $\beta$ -HB), TG, and cholesterol concentrations were determined using commercially available kits (Roche Diagnostics, Mannheim, Germany and Wako Chemicals, Neuss, Germany).

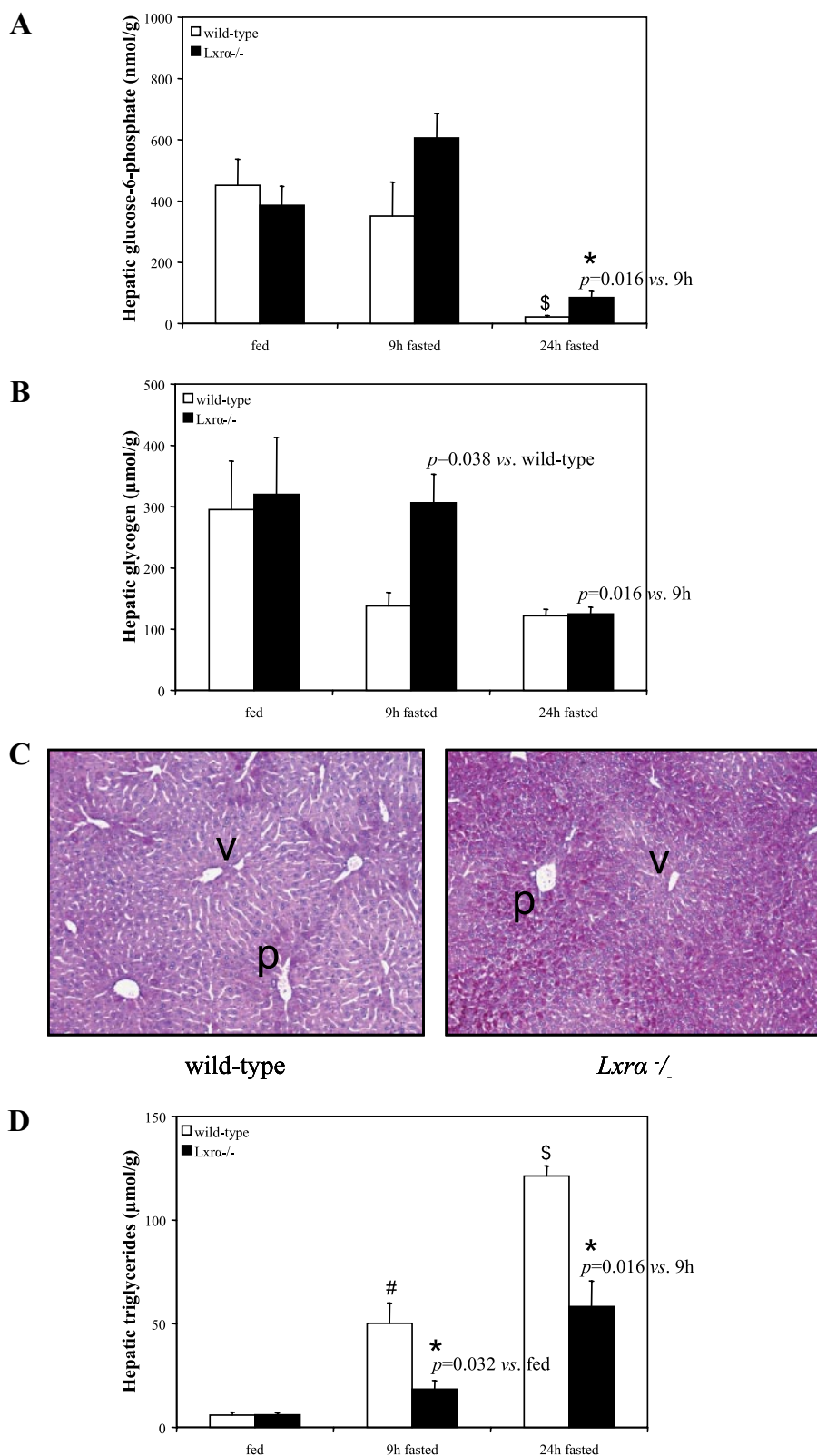
**Hepatic Metabolite Content and Gene Expression Levels**—Livers were quickly removed, weighed, freeze-clamped, and stored at  $-80^{\circ}\text{C}$ . A small piece of liver was fixed in 4% formalin in phosphate-buffered saline for histological analysis. Blood was centrifuged ( $4000 \times g$  for 10 min at  $4^{\circ}\text{C}$ ), and plasma was stored at  $-20^{\circ}\text{C}$ . Frozen liver was homogenized in ice-cold saline. Hepatic TG concentrations were analyzed using a commercially available kit (Roche Diagnostics) after lipid extraction according to Bligh and Dyer (18). Hepatic G6P and glycogen content were determined as described previously (19, 20). In addition, hepatic glycogen disposition was visualized by periodic acid-Schiff staining of 3- $\mu\text{m}$ -thick liver slices. RNA was extracted from frozen liver using TRI reagent (Sigma) and subsequently converted into cDNA by a reverse transcription procedure using Moloney murine leukemia virus transcriptase and random primers according to the manufacturer's protocol. For real-time PCR, cDNA was amplified using the appropriate primers and probes. Primer and probe sequences for *18S*, ATP binding cassette a1/g5/g8 (*Abca1/g5/g8*), carbohydrate response element binding protein (*Chrebp*), fatty acid synthase (*Fas*), fructose-1,6-biphosphatase 1 (*Fbp1*), glucose-6-phosphate hydrolase (*G6ph*), glucose-6-phosphate translocase (*G6pt*), peroxisome proliferator activated receptor gamma co-

activator 1 alpha (*Pgc-1 $\alpha$* ), phosphoenolpyruvate carboxykinase (*Pepck*), pyruvate dehydrogenase kinase 4 (*Pdk4*), stearyl-CoA desaturase 1 (*Scd1*), and sterol regulatory element binding protein 1c (*Srebp-1c*) have been published previously (www.LabPediatricsRug.nl). For acetyl-CoA carboxylase 1 (*Acc1*) the following primer/probes were used: sense, CCA TCC AAA CAG AGG GAA CAT C; antisense, CTA CAT GAG TCA TGC CAT AGT GGT T; probe, ACG CTA AAC AGA ATG TCC TTT GCC TCC AAC (accession number NM\_133360.2). For acetyl-CoA carboxylase 2 (*Acc2*): sense, CCC AGG AGG CTG CAT TGA; antisense, AGA CAT GCT GGG CCT CAT AGT A; probe, CAC AAG TGA TCC TGA ATC TCA CGC GC (accession number NM\_133904.1). All mRNA levels were normalized for *18S* expression.

**In Vivo Flux Measurements**—Mice were equipped with a permanent catheter in the right atrium via the jugular vein (21) and were allowed a recovery period of at least 3 days. After the recovery period, the mice were placed in experimental cages and were fasted from 11 p.m. to 8 a.m. with drinking water available. All infusion experiments were performed in conscious, unrestrained mice. To determine hepatic carbohydrate fluxes, mice were infused with a solution containing [ $U$ - $^{13}\text{C}$ ]glucose (7  $\mu\text{M}$ ), [ $2$ - $^{13}\text{C}$ ]glycerol (82  $\mu\text{M}$ ), [ $1$ - $^2\text{H}$ ]galactose (17  $\mu\text{M}$ ), and paracetamol (1 mg/ml) during 6 h at an infusion rate of 0.6 ml/h as described previously (22, 23). Blood glucose concentrations were measured every 30 min. Blood and urine spots were collected every 60 min on filter paper. In total, 80–90  $\mu\text{l}$  of blood was withdrawn per animal from the tail vein during these experiments.

Hyperinsulinemic euglycemic clamps were performed in a separate group of mice as described earlier (8). Mice were fasted from 11 p.m. to 8 a.m. the next day with drinking water available. During 6 h, they were infused with two solutions. The first solution contained bovine serum albumin (1% w/v, Sigma), somatostatin (40  $\mu\text{g/ml}$ , UCB, Breda, The Netherlands), insulin (110 milliunits/ml, Actrapid, Novo Nordisk, Bagsvaerd, Denmark), glucose (111 mM), and [ $U$ - $^{13}\text{C}$ ]glucose (33 mM, 99%  $^{13}\text{C}$  atom percent excess, Cambridge Isotope Laboratories, Andover, MA) and was infused at a rate of 0.135 ml/h. The second solution consisted of glucose (111 mM) containing [ $U$ - $^{13}\text{C}$ ]glucose (33 mM). The infusion rate of this solution was variable to maintain euglycemia. Blood glucose concentrations were measured every 15 min. Every 30 min, a bloodspot was collected. In total, 150–170  $\mu\text{l}$  of blood was withdrawn per animal from the tail vein during these experiments.





**FIGURE 1. Fasting response in *Lxra*<sup>-/-</sup> mice and their wild-type littermates.** Mice were fasted for 0, 9, and 24 h. G6P and glycogen content in liver were determined by an enzymatic assay. Hepatic glycogen was visualized in liver slices of 9-h fasted mice by periodic acid-Schiff staining. Hepatic TG content was analyzed using a commercial available kit after lipid extraction. *A*, hepatic G6P content; *B*, hepatic glycogen content; *C*, hepatic glycogen content and localization in 9-h fasted mice. *P*, periportal; *V*, perivenous. *D*, hepatic TG content. Open bars, wild-type mice; filled bars, *Lxra*<sup>-/-</sup> mice. Values represent means  $\pm$  S.E. for  $n = 4-6$ ; #,  $p < 0.05$  9-h fasted versus fed; \$,  $p < 0.05$  24-h fasted versus 9-h fasted; \*,  $p < 0.05$  *Lxra*<sup>-/-</sup> versus wild-type (Mann-Whitney *U* test,  $p$  value adjusted for multiple comparisons).

Analytical procedures for extraction of glucose from blood spots, derivatization of the extracted compounds and gas chromatography-mass spectrometry measurements of derivatives were performed according to van Dijk *et al.* (22–24). From this, hepatic carbohydrate fluxes were calculated using mass isotopomer distribution analysis as previously described (22, 23, 25). Supplemental Fig. S1 depicts the isotopic model used. To balance input and output of hepatic G6P, minor adaptations were made to the published equations (26). The equations are given in supplemental Table S1. Glucose production and metabolic clearance rates during hyperinsulinemic euglycemic clamps were calculated according to Grefhorst *et al.* (8).

**Statistics**—All data are presented as mean values  $\pm$  S.E. Statistical analysis was performed using SPSS for Windows software (SPSS 12.02, Chicago, IL). Analysis of data obtained in *Lxra*<sup>-/-</sup> versus wild-type mice was assessed by Mann-Whitney *U* test for plasma and liver parameters. *In vivo* flux data were analyzed by analysis of variance for repeated measurements. The null hypothesis was rejected at the 0.05 level of probability, except for the fasting-refeeding experiments, where this  $p$  value was adjusted for multiple comparisons.

## RESULTS

**The Fasting Response Is Hampered in *Lxra*<sup>-/-</sup> Mice**—We compared the changes in metabolic parameters in fasted *Lxra*<sup>-/-</sup> mice and wild-type littermate controls. Upon fasting, blood glucose and plasma insulin concentrations decreased while plasma NEFA and  $\beta$ -hydroxybutyrate concentrations increased, without differences between *Lxra*<sup>-/-</sup> and wild-type mice (Table 1). Plasma TG concentrations increased upon fasting in both genotypes while plasma cholesterol concentrations were not affected. Compared with wild-type mice, hepatic G6P content tended

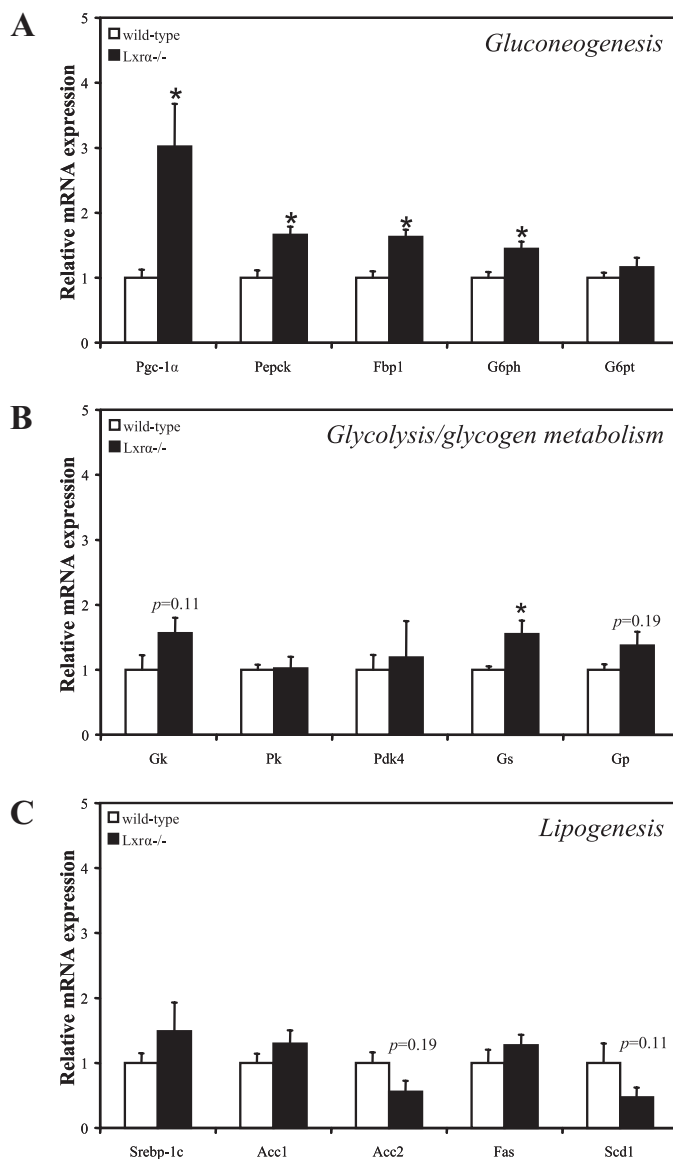
## Lxra Mediates the Hepatic Response to Fasting

to be higher in 9-h fasted  $Lxra^{-/-}$  mice (Fig. 1A, +73%,  $p = 0.26$ ). Twenty-four hours of fasting decreased hepatic G6P content in both phenotypes, but this drop was less pronounced in  $Lxra^{-/-}$  mice. Hepatic glycogen content decreased upon fasting in both groups (Fig. 1B). However, in wild-type mice hepatic glycogen content already reached its lowest level after a 9-h fast, whereas in 9-h fasted  $Lxra^{-/-}$  mice it was similar to what observed in the fed state. Histological analysis revealed that the glycogen in the 9-h fasted  $Lxra^{-/-}$  mice was mainly located in the periportal zone (Fig. 1C). After 24 h of fasting, hepatic glycogen stores were similarly depleted in both genotypes (Fig. 1B). Hepatic TG content increased upon fasting, but to a markedly less extent in  $Lxra^{-/-}$  mice compared with wild-type controls (Fig. 1D).

Gluconeogenic flux plays an essential role in glycogen accumulation (27) and hepatic gluconeogenic gene expression, e.g. of *Pepck* and *G6pase*, has been shown to be decreased upon *Lxr* activation (9–11). We therefore determined whether the increased hepatic glycogen content in the 9-h fasted  $Lxra^{-/-}$  mice was paralleled by an increased expression of genes encoding enzymes involved in hepatic gluconeogenesis. Compared with wild-type mice, hepatic expression of *Pgc-1 $\alpha$* , *Pepck*, *Fbp1*, and *G6ph* (encoding G6P hydrolase, one component of the multiprotein complex G6Pase) were all increased in 9-h fasted  $Lxra^{-/-}$  mice (Fig. 2A). Expression of genes encoding other major enzymes involved in hepatic carbohydrate metabolism (*G6pt*, *Gk*, *Pk*, *Pdk4*, and *Gp*, except for *Gs*, Fig. 2, A and B) was not affected by *Lxra* deficiency. Moreover, the lipogenic gene expression profile was similar in 9-h fasted wild-type and  $Lxra^{-/-}$  mice, except for a reduction of *Acc2* and *Scd1* expression (Fig. 2C).

**Impaired Hepatic G6P Metabolism in 9-h Fasted  $Lxra^{-/-}$  Mice Is Associated with Decreased Glucose Turnover and Increased Hepatic G6P Content**—A 9-h fast uncovered major differences in hepatic adaptive response between wild-type and  $Lxra^{-/-}$  mice. To determine whether the increased gluconeogenic gene expression was a cause of the observed differences in hepatic glycogen and G6P content between 9-h fasted wild-type and  $Lxra^{-/-}$  mice, we determined glucose turnover, disposal, and individual hepatic carbohydrate fluxes using stable isotope techniques (22, 28). During the infusion of the stable isotopes, blood glucose concentrations were lower in  $Lxra^{-/-}$  mice compared with wild-type littermates (Fig. 3A). Steady-state isotope enrichment was reached from 3 h of infusion onwards. Isotope dilution data during this steady-state situation are shown in Table 2. Glucose cycling and endogenous glucose production were reduced in  $Lxra^{-/-}$  mice compared with their wild-type littermates (Fig. 3B), resulting in a decreased total glucose production. Metabolic glucose clearance was similar in both groups of mice (Fig. 3C).

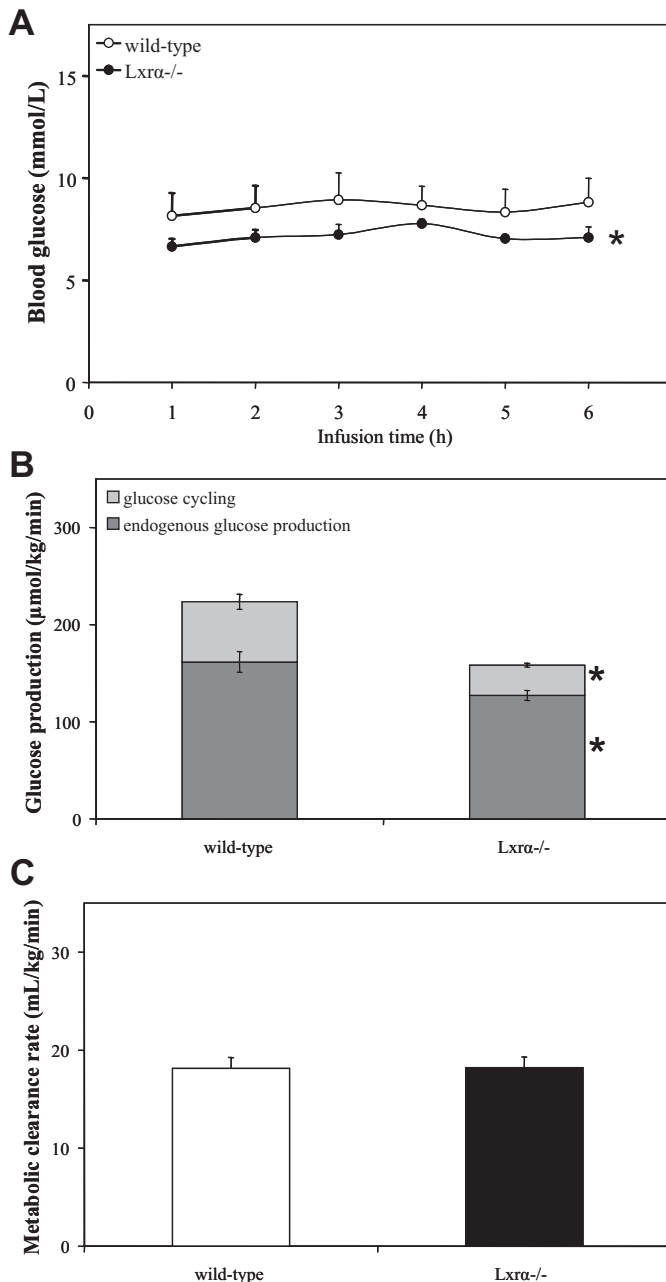
Gluconeogenic flux, e.g. *de novo* synthesis of G6P was not affected by *Lxra* deficiency (Table 3). In addition, the compartmentation of newly synthesized G6P toward glucose ( $86 \pm 1\%$  in both  $Lxra^{-/-}$  and wild-type mice) and glycogen ( $14 \pm 1\%$  in both  $Lxra^{-/-}$  and wild-type mice) was comparable in both genotypes. However, glucose phosphorylation (glucokinase flux), dephosphorylation (glucose-6-phosphatase flux), glycogen synthesis (glycogen synthase flux), and glycogen breakdown



**FIGURE 2. Hepatic gene expression levels in 9-h fasted  $Lxra^{-/-}$  mice and their wild-type littermates.** A, gluconeogenic gene expression; B, glycolytic gene expression; and C, lipogenic gene expression. *Acc*, acetyl CoA carboxylase; *Fas*, fatty acid synthase; *Fbp1*, fructose-1,6-bisphosphatase 1; *G6ph*, glucose-6-phosphate hydrolase; *G6pt*, glucose-6-phosphate translocase; *Gk*, glucokinase; *Gp*, glycogen phosphorylase; *Gs*, glycogen synthase; *Pepck*, phosphoenolpyruvate carboxykinase; *Pdk4*, pyruvate dehydrogenase kinase 4; *Pgc-1 $\alpha$* , peroxisome proliferator-activated receptor  $\gamma$  co-activator 1 $\alpha$ ; *Pk*, pyruvate kinase; *Scd1*, stearoyl-CoA desaturase 1; *Srebp-1c*, sterol regulatory element binding protein 1c. Open bars, wild-type mice; filled bars,  $Lxra^{-/-}$  mice. Values represent means  $\pm$  S.E. for  $n = 5$ ; \*,  $p < 0.05$   $Lxra^{-/-}$  versus wild-type (Mann-Whitney U test).

(glycogen phosphorylase flux) were reduced in  $Lxra^{-/-}$  mice compared with wild-type mice (Table 3). G6P turnover and glucose balance were reduced in  $Lxra^{-/-}$  mice compared with wild-type littermates, while glycogen balance tended to be less negative in  $Lxra^{-/-}$  mice (Fig. 4).

**Hepatic and Peripheral Insulin Sensitivity Are Maintained in  $Lxra^{-/-}$  Mice**—Insulin is a major regulator of carbohydrate metabolism. Although plasma insulin concentrations did not differ between 9-h fasted wild-type and  $Lxra^{-/-}$  mice (Table 1), we questioned whether insulin sensitivity of hepatic and peripheral glucose metabolism was altered in  $Lxra^{-/-}$  mice.



**FIGURE 3. Whole body glucose fluxes in 9-h fasted *Lxra*<sup>-/-</sup> mice and their wild-type littermates during steady state infusion ( $t = 180$ – $360$  min).** These parameters were calculated using the equations listed in supplemental Table S1. **A**, blood glucose concentrations during isotope infusion. Open dots, wild-type mice; filled dots, *Lxra*<sup>-/-</sup> mice. **B**, total glucose production and contribution of endogenous glucose production (dark gray bars) and glucose cycling (light gray bars). **C**, metabolic glucose clearance rates. Open bars, wild-type mice; filled bars, *Lxra*<sup>-/-</sup> mice. Values represent means  $\pm$  S.E. for  $n = 6$ ; \*,  $p < 0.05$  *Lxra*<sup>-/-</sup> versus wild-type (analysis of variance for repeated measurements).

We therefore performed hyperinsulinemic euglycemic clamps in 9-h fasted conscious, unrestrained mice. Steady-state isotope enrichment and euglycemia (Fig. 5A) were reached within 3 h of infusion. The glucose infusion rates to maintain euglycemic conditions (Fig. 5B) did not differ between the two genotypes, indicative for unaffected whole body insulin-sensitivity in *Lxra*<sup>-/-</sup> mice compared with wild-type littermates. Hepatic insulin sensitivity was not affected in *Lxra*<sup>-/-</sup> mice. Hyperin-

**TABLE 2**

**Primary isotopic parameters during steady state infusion ( $t = 180$ – $360$  min) in 9-h fasted *Lxra*<sup>-/-</sup> mice and their wild-type littermates**

The parameters were calculated using the equations listed in supplemental Table S1. Values represent means  $\pm$  S.E. for  $n = 6$ .

	Wild-type	<i>Lxra</i> <sup>-/-</sup>
<b>Isotope dilution</b>		
d(glc)	0.016 $\pm$ 0.001	0.019 $\pm$ 0.001 <sup>a</sup>
d(UDPglc)	0.141 $\pm$ 0.008	0.164 $\pm$ 0.006 <sup>a</sup>
<b>Isotope exchange</b>		
c(glc)	0.27 $\pm$ 0.02	0.19 $\pm$ 0.01 <sup>a</sup>
c(UDPglc)	0.15 $\pm$ 0.03	0.11 $\pm$ 0.01
<b>MIDA analysis</b>		
f(glc)	0.66 $\pm$ 0.03	0.71 $\pm$ 0.02
f(UDPglc)	0.54 $\pm$ 0.02	0.52 $\pm$ 0.01

<sup>a</sup> $p < 0.05$  *Lxra*<sup>-/-</sup> versus wild-type (analysis of variance for repeated measurements). For abbreviations see supplemental Table S1.

**TABLE 3**

**Individual fluxes comprising hepatic G6P metabolism during steady-state infusion ( $t = 180$ – $360$  min) in 9-h fasted *Lxra*<sup>-/-</sup> mice and their wild-type littermates**

The individual fluxes were calculated using the equations listed in supplemental Table S1. Values represent means in micromoles/kg/min  $\pm$  S.E. for  $n = 6$ .

	Wild-type	<i>Lxra</i> <sup>-/-</sup>
Gluconeogenic flux	109 $\pm$ 6	98 $\pm$ 4
Glucokinase flux	75 $\pm$ 9	39 $\pm$ 3 <sup>a</sup>
Glucose-6-phosphatase flux	223 $\pm$ 16	158 $\pm$ 6 <sup>a</sup>
Glycogen synthase flux	45 $\pm$ 4	34 $\pm$ 2 <sup>a</sup>
Glycogen phosphorylase flux	71 $\pm$ 7	48 $\pm$ 4 <sup>a</sup>

<sup>a</sup> $p < 0.05$  *Lxra*<sup>-/-</sup> versus wild-type (analysis of variance for repeated measurements).

sulinemia resulted in a 41 and 51% reduction of hepatic glucose production in *Lxra*<sup>-/-</sup> and wild-type mice, respectively (compare Fig. 5C with Fig. 3B). In addition, peripheral insulin sensitivity was not affected by *Lxra* deficiency, because the MCR was increased to 406% in *Lxra*<sup>-/-</sup> mice and 378% in wild-type littermates (compare Figs. 5D with 3C).

**Carbohydrate Refeeding Affects Hepatic Lipogenesis and Gene Transcription Independent of LXR $\alpha$** —We also determined whether there are indications for glucose-mediated LXR activation. Therefore, plasma and liver metabolite concentrations were assessed in *Lxra*<sup>-/-</sup> and wild-type mice that were refed a carbohydrate rich diet following a 24-h fast (Table 4). Blood glucose and plasma insulin, NEFA, and  $\beta$ -hydroxybutyrate concentrations were comparable in both groups of carbohydrate-refed mice. Plasma TG concentrations were lower in carbohydrate-refed *Lxra*<sup>-/-</sup> mice compared with wild-type mice, whereas plasma cholesterol concentrations were similar. Hepatic G6P and glycogen content were increased in carbohydrate-refed mice compared to mice that had been fasted for 24 h (Fig. 2, A and B), but no differences were observed between the two genotypes (Table 4). Hepatic TG content was lower in carbohydrate-refed *Lxra*<sup>-/-</sup> mice ( $p = 0.052$ ).

In both groups of mice, carbohydrate refeeding increased expression of *Gk*, *Pk*, and *Gp*, while *Pdk4* expression was decreased. *Chrebp* and *Gs* expression were not affected by carbohydrate refeeding (Fig. 6A). Expression of *Srebp-1c*, *Acc1*, *Fas*, and *Scd1* was clearly induced in carbohydrate-refed wild-type mice, but this response was less pronounced in *Lxra*<sup>-/-</sup> mice. *Acc2* expression was not affected by carbohydrate refeeding (Fig. 6B). In both wild-type and *Lxra*<sup>-/-</sup> mice, expression of the LXR target genes *Abca1*, *Abcg5*, and *Abcg8* was not induced by carbohydrate-refeeding (Fig. 6C).

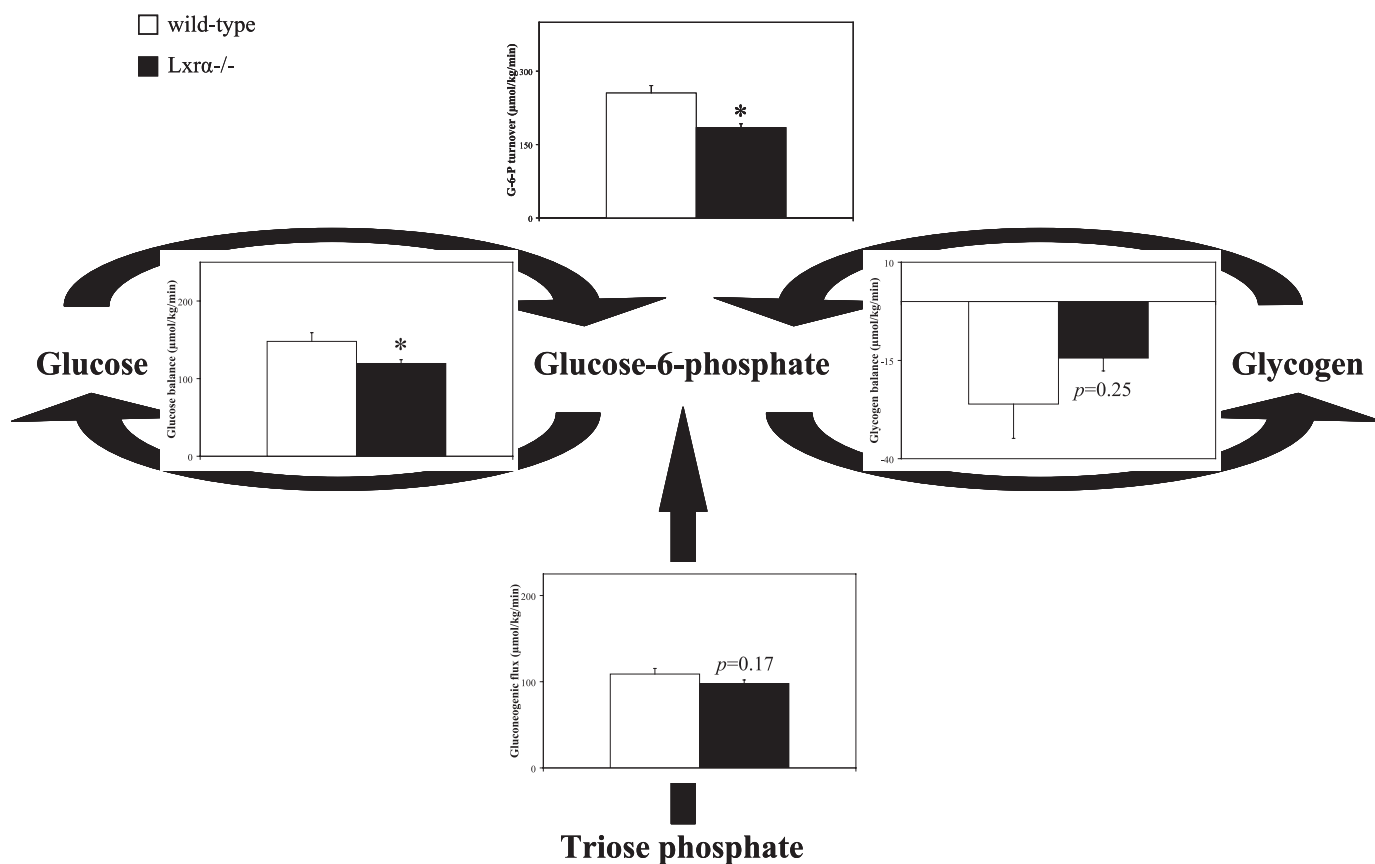


FIGURE 4. Hepatic glucose balance, glycogen balance, G6P turnover, and gluconeogenic flux in 9-h fasted *Lxrα*<sup>-/-</sup> mice and their wild-type littermates during steady state infusion ( $t = 180$ – $360$  min). Glucose and glycogen balances were calculated from the difference between input (glucokinase flux for glucose balance and glycogen synthase flux for glycogen balance) and output (glucose-6-phosphatase flux for glucose balance and glycogen phosphorylase flux for glycogen balance). G6P turnover was calculated from the total G6P input (sum of gluconeogenic flux, glucokinase flux, and glycogen phosphorylase flux). Open bars, wild-type mice; filled bars, *Lxrα*<sup>-/-</sup> mice. Values represent means  $\pm$  S.E. for  $n = 6$ ; \*,  $p < 0.05$  *Lxrα*<sup>-/-</sup> versus wild-type (analysis of variance for repeated measurements).

## DISCUSSION

LXRs act as cholesterol sensors that control transcription of genes involved in cellular cholesterol and lipid homeostasis. Lipid and carbohydrate metabolism are tightly linked and strongly regulated to ensure an adequate control of whole body energy metabolism. LXR regulates transcription and activity of the glucose-sensing lipogenic transcription-factor ChREBP (4), which strongly suggest a physiological role of LXR in hepatic carbohydrate metabolism in the postprandial state. It is known that LXR activation results in hepatic steatosis (5, 29). On the other hand, prolonged fasting is also associated with hepatic lipid accumulation (30). These lines of evidence prompted us to study the role of hepatic LXR during fasting and refeeding. LXR $\alpha$  is considered to be the major isoform regulating lipogenic gene expression in the liver. Therefore, we subjected *Lxrα*<sup>-/-</sup> mice (17) to fasting and refeeding protocols, and we applied sophisticated stable isotope techniques to quantify hepatic carbohydrate fluxes *in vivo* in these mice.

We are the first to show that *Lxrα* plays an important role in the feeding-to-fasting transition. *Lxrα* deficiency results in an impaired fasting response, indicated by a delayed fasting-induced hepatic glycogen depletion and increased hepatic G6P content in 9-h fasted *Lxrα*<sup>-/-</sup> mice compared with wild-type

littermates. Moreover, the *Lxrα*<sup>-/-</sup> mice accumulated less hepatic TG upon fasting.

Expression of gluconeogenic genes was increased in 9-h fasted *Lxrα*<sup>-/-</sup> mice compared with wild-type littermates. This is in agreement with the decreased expression of *Pgc-1α*, *G6pase*, and *Pepck* upon pharmacological LXR activation (9–11). However, evaluation of hepatic carbohydrate fluxes in 9-h fasted mice revealed that the induction of gluconeogenic gene expression in *Lxrα*<sup>-/-</sup> mice was not paralleled by an increased gluconeogenic flux. Thus, there is a discrepancy between gene expression levels and gluconeogenic flux *in vivo* (8). This indicates that other factors such as precursor availability (31, 32) and post-transcriptional modification of enzymes are important determinants that control hepatic carbohydrate fluxes *in vivo*.

Glucose phosphorylation and dephosphorylation as well as glycogen synthesis and breakdown were reduced in *Lxrα*<sup>-/-</sup> mice compared with wild-type littermates. Thus, instead of an altered *de novo* synthesis of G6P the interconversions of G6P, glucose and glycogen were clearly affected in 9-h fasted *Lxrα*<sup>-/-</sup> mice. The net effect of the lower glycogen synthesis (–24%) and breakdown (–32%) fluxes in *Lxrα*<sup>-/-</sup> mice was a less negative glycogen balance, supporting the delayed glycogen depletion observed upon fasting in the *Lxrα*-deficient mice.



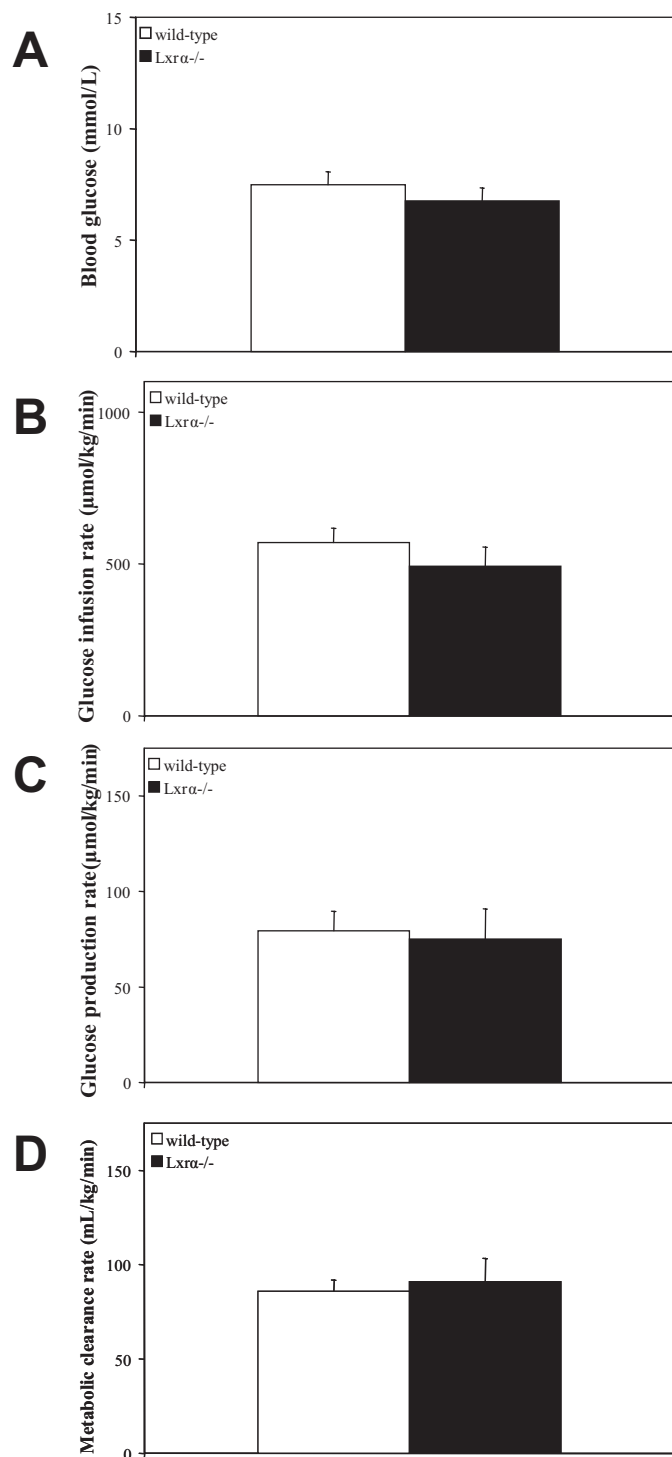


FIGURE 5. Glucose metabolism under hyperinsulinemic euglycemic clamp conditions in 9-h fasted *Lxra*<sup>-/-</sup> mice and their wild-type littermates during steady state infusion ( $t = 180\text{--}360$  min). A, blood glucose concentrations. B, glucose infusion rates required to maintain euglycemia. C, endogenous glucose production rates. D, metabolic glucose clearance rates. Open bars, wild-type mice; filled bars, *Lxra*<sup>-/-</sup> mice. Values represent means  $\pm$  S.E. for  $n = 5$ .

The remaining glycogen was located in the periportal zone. It is known that upon fasting, glycogen is initially degraded to G6P in periportal hepatocytes. In perivenous hepatocytes, glycogen is predominantly broken down into pyruvate and hence released as lactate (reviewed in Ref. 33). Thus in the livers of 9-h

TABLE 4

Plasma and liver parameters upon refeeding in *Lxra*<sup>-/-</sup> mice and their wild-type littermates

Mice were fasted for 24 h and refed a carbohydrate rich diet during 24 h. Blood glucose concentrations were measured using a EuroFlash glucose meter. Plasma insulin, NEFA,  $\beta$ -HB, TG, and cholesterol concentrations were determined using commercially available kits. Hepatic G6P and glycogen content were determined using an enzymatic assay. Hepatic TG content was analyzed using a commercial available kit after lipid extraction. Values represent means  $\pm$  S.E. for  $n = 5\text{--}6$ .

	Wild-type	<i>Lxra</i> <sup>-/-</sup>
Blood glucose (mM)	9.5 $\pm$ 0.5	9.8 $\pm$ 0.6
Plasma insulin (ng/mL)	1.66 $\pm$ 0.49	2.74 $\pm$ 0.66
Plasma NEFA (mM)	0.34 $\pm$ 0.01	0.30 $\pm$ 0.02
Plasma $\beta$ -HB (mM)	0.11 $\pm$ 0.01	0.12 $\pm$ 0.01
Plasma TG (mM)	3.00 $\pm$ 0.18	1.78 $\pm$ 0.23 <sup>a</sup>
Plasma cholesterol (mM)	3.7 $\pm$ 0.2	3.3 $\pm$ 0.1
Hepatic G6P (nmol/g)	347 $\pm$ 23	359 $\pm$ 41
Hepatic glycogen ( $\mu$ mol/g)	1121 $\pm$ 81	1088 $\pm$ 91
Hepatic TG ( $\mu$ mol/g)	18.1 $\pm$ 1.5	13.0 $\pm$ 1.6

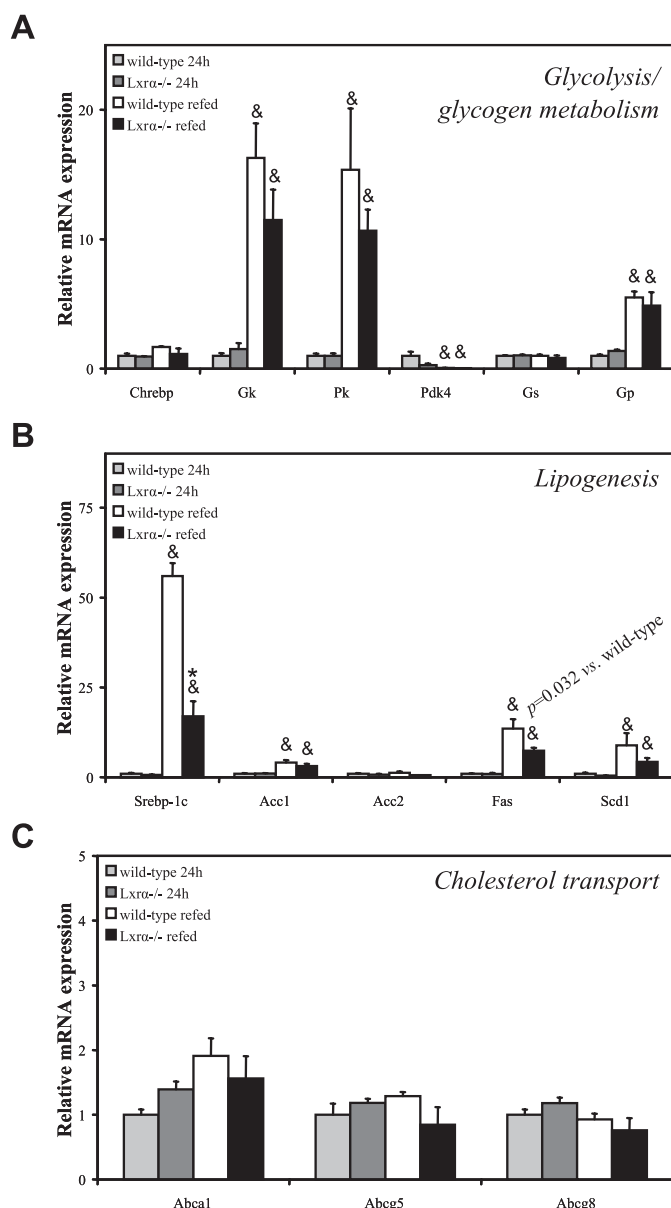
<sup>a</sup>  $p < 0.05$  *Lxra*<sup>-/-</sup> versus wild-type (Mann-Whitney *U*-test).

fasted *Lxra*<sup>-/-</sup> mice, less glycogen was broken down, contributing to the reduced G6P turnover observed in these mice. The changes in G6P and glycogen metabolism were not secondary to changes in hepatic gluconeogenesis (27, 34), because neither the gluconeogenic flux nor the partitioning of newly synthesized G6P toward glucose and glycogen was affected by *Lxra* deficiency. In addition, the net effect of reduced glucokinase and glucose-6-phosphatase fluxes was a reduction in endogenous glucose production and glucose cycling.

Glycogen synthesis and breakdown are regulated by several factors, including insulin. Although insulin concentrations were comparable in 9-h fasted *Lxra*<sup>-/-</sup> mice and their wild-type littermates, hepatic insulin sensitivity could have been altered by *Lxra* deficiency, explaining the differences observed in hepatic G6P and glycogen content as well as their interconversions. Hepatic and peripheral insulin sensitivity were determined in 9-h fasted *Lxra*<sup>-/-</sup> mice and their wild-type littermates using hyperinsulinemic euglycemic clamps. Insulin sensitivity of both hepatic glucose production and peripheral glucose disposal was not affected by *Lxra* deficiency. Although LXR agonists have been implicated as potential insulin sensitizers (9, 10, 12), our data do not support a direct role of LXR as a potential mediator of hepatic and peripheral insulin sensitivity (8). However, many of the studies performed on the role of LXR are based on pharmacological activation. In the *Lxra*<sup>-/-</sup> mice there may be some adaptations that prevent the endogenous ligand from increasing, or there may be additional systems that compensate for the *Lxra* deficiency. The reduced hepatic carbohydrate fluxes could also be a result from an altered reliance on glucose versus fatty acids and/or a differential energy demand in the *Lxra*<sup>-/-</sup> mice during the feeding-to-fasting transition. In addition to the delay in glycogen depletion observed upon fasting in the *Lxra*<sup>-/-</sup> mice, these mice accumulated remarkably less TG. Gene expression analysis provided indications for an increase in hepatic fatty acid oxidation in fasted *Lxra*<sup>-/-</sup> mice, which could explain this remarkable reduction in hepatic TG accumulation (data not shown). However, additional *in vivo* studies are required to determine the physiological relevance of these observations.

Finally, we explored the role of LXR $\alpha$  in glucose-induced hepatic lipogenesis. Upon refeeding, hepatic TG content was lower, and plasma TG levels were reduced in *Lxra*<sup>-/-</sup> mice





**FIGURE 6. Hepatic gene expression levels upon fasting and refeeding in *Lxra*<sup>-/-</sup> mice and their wild-type littermates.** Mice were fasted for 24 h and refeed a carbohydrate rich diet during 24 h. *A*, glycolytic gene expression; *B*, lipogenic gene expression; *C*, cholesterol transporter gene expression. *Abca1/g5/g8*, Atp binding cassette a1/g5/g8; *Acc*, acetyl CoA carboxylase; *Chreb*, carbohydrate response element binding protein; *Fas*, fatty acid synthase; *Gk*, glucokinase; *Gp*, glycogen phosphorylase; *Gs*, glycogen synthase; *Pdk4*, pyruvate dehydrogenase kinase 4; *Pk*, pyruvate kinase; *Scd1*, stearoyl-CoA desaturase 1; *Srebp-1c*, sterol regulatory element binding protein 1c. Light gray bars, 24-h fasted wild-type mice; dark gray bars, 24-h fasted *Lxra*<sup>-/-</sup> mice; open bars, refeed wild-type mice; filled bars, refeed *Lxra*<sup>-/-</sup> mice. Values represent means  $\pm$  S.E. for  $n = 5$ ; \*,  $p < 0.05$  refeed versus 24-h fasted; \*,  $p < 0.05$  *Lxra*<sup>-/-</sup> versus wild-type (Mann-Whitney *U* test,  $p$  value adjusted for multiple comparisons).

compared with wild types. Quite strikingly, no differences in *Chreb* expression were observed between *Lxra*<sup>-/-</sup> and wild-type mice. This is in contrast to observations by Cha and Repa (4), which suggested that CHREBP is a downstream target of LXR $\alpha$ . However, carbohydrate refeeding resulted in a less pronounced induction of *Srebp-1c* and other lipogenic gene expression in the *Lxra*<sup>-/-</sup> mice compared with the wild-types. Considering our observation that *Chreb* and *Pk* expression

were similar in carbohydrate-refed *Lxra*<sup>-/-</sup> and wild-type mice, we conclude that the blunted lipogenic response in carbohydrate-refed *Lxra*<sup>-/-</sup> mice resulted from the reduced SREBP-1c activity secondary to *Lxra* deficiency. Apparently, the relationship between LXR, ChREBP, and SREBP-1c on the one hand and hepatic TG metabolism on the other hand requires further investigation.

Recent *in vitro* studies have shown that glucose is able to bind and activate hepatic LXR (13), suggesting that LXR may act as a putative hepatic "glucose sensor." However, the physiological relevance of glucose sensing by LXR has been debated (14–16) and therefore required further investigation. In the studies performed by Mitro *et al.* (13), the expression of the cholesterol transporters that are direct LXR targets, *e.g.* *Abca1* and *Abcg1*, only marginally increased upon carbohydrate-refeeding, whereas lipogenic mRNA expression was clearly induced.

We confirmed that the expression of the classic LXR-target genes *Abca1*, *Abcg5*, and *Abcg8* was not affected by carbohydrate-refeeding in *Lxra*<sup>-/-</sup> mice. Thus, the effect of carbohydrate refeeding on hepatic lipogenic gene expression was different from that on expression of the cholesterol transporters *Abca1*, *Abcg5*, and *Abcg8*. Similar results have been obtained by Denechaud *et al.* (16), who showed no induction of hepatic *Abcg1* and *Abca1* mRNA expression in carbohydrate-refed mice, whereas lipogenic gene expression was induced. Moreover, in contrast to the blunted induction of lipogenic gene expression, *Abcg1* and *Abca1* expression was not affected in carbohydrate-refed *Lxra*<sup>-/-</sup> mice compared with wild-type controls (16). Taken together, these and our data provide strong evidence that carbohydrate refeeding does not induce hepatic gene expression via LXR and, therefore, question the physiological relevance of glucose sensing by hepatic LXR *in vivo*.

In summary, our data identify LXR $\alpha$  as an important player in control of metabolic adaptation during the feeding-to-fasting transition but question the physiological relevance of glucose sensing by hepatic LXR. In addition to its regulatory role in cholesterol, lipid, and glucose metabolism to ensure energy storage in the postprandial state, LXR $\alpha$  seems to facilitate the release of stored energy upon fasting. Under these conditions, LXR $\alpha$  not only mediates TG accumulation, but also controls hepatic G6P and glycogen deposition, because it determines the partitioning and turnover of these energy-bearing molecules, possibly to fulfill the liver's demand for these metabolites.

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*Legend Supplementary Table 1*

d(glc), fractional contribution of infused glucose to blood glucose;  $M_6(\text{glc})_{\text{infusate}}$ , mole percent enrichments (MPE) of  $[\text{U-}^{13}\text{C}]$ glucose in the infusate;  $M_6(\text{glc})_{\text{blood}}$ , MPE of  $[\text{U-}^{13}\text{C}]$ glucose in blood; d(UDPglc), fractional contribution of infused galactose to UDPglucose;  $M_1(\text{gal})_{\text{infusate}}$ , MPE of  $[\text{1-}^2\text{H}]$ galactose in the infusate;  $M_1(\text{pGlcUA})_{\text{urine}}$ , MPE of  $[\text{1-}^2\text{H}]$ -UDPglucose measured in urinary Par-GlcUA; c(glc), fractional contribution of blood glucose to UDP-glucose formation;  $M_6(\text{pGlcUA})_{\text{urine}}$ , MPE of  $[\text{U-}^{13}\text{C}]$ - UDPglucose measured in urinary Par-GlcUA; c(UDPglc), fractional contribution of UDPglucose to blood glucose formation;  $M_1(\text{glc})_{\text{blood}}$ , MPE of  $[\text{1-}^2\text{H}]$ - glucose in blood,  $M_6(\text{pGlcUA})_{\text{urine}}$ , MPE of  $[\text{U-}^{13}\text{C}]$ -UDPglucose measured in urinary Par-GlcUA; f(glc), fractional contribution of newly synthesized glucose to blood glucose;  $M_2(\text{glc})_{\text{blood}}$ , MPE of  $[\text{1}^3\text{C}_2]$ -glucose in blood;  $M_2(\text{FBP:MIDA})\text{glc}$ , theoretical MPE of  $[\text{1}^3\text{C}_2]$ -Fructose 1,6 biphosphate, calculated by MIDA using  $^{13}\text{C}$ -enrichment data of glucose; f(UDPglc), fractional contribution of newly synthesized glucose to UDPglc pool;  $M_2(\text{pGlcUA})_{\text{urine}}$ , MPE of  $[\text{1}^3\text{C}_2]$ -UDPglc, sampled as urinary Par-GlcUA;  $M_2(\text{FBP:MIDA})\text{pGlcUA}$ , theoretical MPE of  $[\text{1}^3\text{C}_2]$ -Fructose 1,6 biphosphate, calculated by MIDA using  $^{13}\text{C}$  enrichment data of urinary Par-GlcUA; Ra(glc;whole body), whole body rate of appearance of glucose into the blood glucose pool; Inf(glc), rate of infusion of  $[\text{U-}^{13}\text{C}]$ glucose in  $\mu\text{mol.kg}^{-1}.\text{min}^{-1}$ ; Ra(UDPglc;whole body), whole body rate of appearance of UDPglc; Inf(gal), rate of infusion of  $[\text{1-}^2\text{H}]$ galactose in  $\mu\text{mol.kg}^{-1}.\text{min}^{-1}$ ; MCR(glc), metabolic clearance rate of blood glucose; glc conc, blood glucose concentration in  $\text{mmol.L}^{-1}$ ; Ra(glc;endo), rate of endogenous blood glucose appearance, not corrected for recycling of tracer; Ra(UDPglc;endo), rate of endogenous UDP glucose appearance, not corrected for recycling of tracer; Rr(glc), rate of recycling of glucose tracer; Rr(UDPglc), rate of recycling of UDPglc tracer; totalRa(glc;endo), total endogenous glucose production, including recycling of tracer; totalRa(UDPglc), total endogenous UDPglucose production, including recycling of tracer; UDPglc(glc), rate of UDPglucose conversion into blood glucose; glc(UDPglc), rate of blood glucose conversion into UDPglucose; GNG(glc), rate of gluconeogenesis into blood glucose; GNG(UDPglc), rate of gluconeogenesis into UDPglucose; GNG(glc;indirect), rate of gluconeogenesis into blood glucose indirectly via glycogen; GNG(UDPglc;indirect), rate of gluconeogenesis into UDPglucose indirectly via blood glucose; GNG(glc;direct), rate of gluconeogenesis directly into blood glucose; GNG(UDPglc;direct), rate of gluconeogenesis directly into UDPglucose; GLY(glc), rate of glycogenolysis contributing to blood glucose formation; GLY (UDPglc), rate of glycogenolysis contributing to UDPglucose formation; GNG(G6P), total flux of G6P *de novo* synthesis, corrected for the exchange between blood glucose and UDPglucose pools; GK, glucokinase flux; G6Pase, glucose-6-phosphatase flux; GS, glycogen synthase flux; GP, glycogen phosphorylase flux.

*Supplementary Table 1* Parameters and equations used to calculate hepatic glucose metabolism

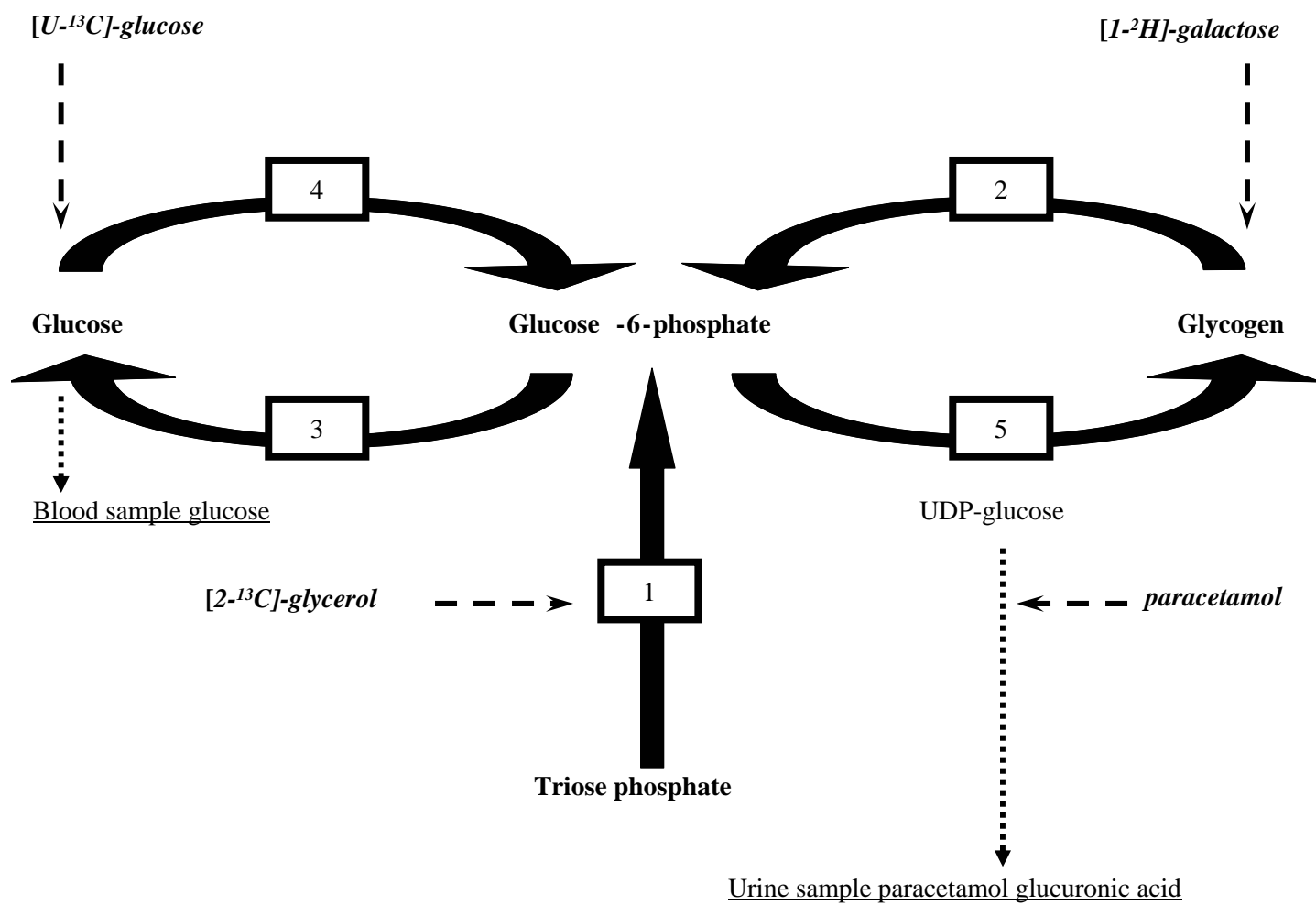
Parameter	Equation
<i>Primary isotopic parameters</i>	
1. d(glc)	$M_6(\text{glc})_{\text{blood}}/M_6(\text{glc})_{\text{infusate}}$
2. d(UDPglc)	$M_1(\text{pGlcUA})_{\text{urine}}/M_1(\text{gal})_{\text{infusate}}$
3. c(glc)	$M_6(\text{pGlcUA})_{\text{urine}}/M_6(\text{glc})_{\text{blood}}$
4. c(UDPglc)	$M_1(\text{glc})_{\text{blood}}/M_1(\text{pGlcUA})_{\text{urine}}$
5. f(glc)	$M_2(\text{glc})_{\text{blood}}/M_2(\text{FBP:MIDA})_{\text{glc}}$
6. f(UDPglc)	$M_2(\text{pGlcUA})_{\text{urine}}/M_2(\text{FBP:MIDA})_{\text{pGlcUA}}$
<i>Whole body glucose metabolism</i>	
7. Ra(glc;whole body)	$\text{Inf}(\text{glc};\text{total})/\text{d}(\text{glc})$
8. Ra(UDPglc;whole body)	$\text{Inf}(\text{gal};\text{total})/\text{d}(\text{UDPglc})$
9. MCR(glc)	$\text{Ra}(\text{glc};\text{whole body})/\text{glc conc}$
10. Ra(glc;endo)	$\text{Ra}(\text{glc};\text{whole body}) - \text{Inf}(\text{gal};\text{total})$
11. Ra(UDPglc;endo)	$\text{Ra}(\text{UDPglc};\text{whole body}) - \text{Inf}(\text{gal};\text{total})$
12. Rr(glc)	$\{c(\text{glc})/[1-c(\text{glc})]\}/\text{Ra}(\text{glc};\text{endo})$
13. Rr(UDPglc)	$\{c(\text{UDPglc})/[1-c(\text{UDPglc})]\}/\text{Ra}(\text{UDPglc};\text{endo})$
14. Total Ra(glc;endo)	$\text{Ra}(\text{glc};\text{endo}) + \text{Rr}(\text{glc})$
15. Total Ra(UDPglc;endo)	$\text{Ra}(\text{UDPglc};\text{endo}) + \text{Rr}(\text{UDPglc})$
16. UDPglc(glc)	$c(\text{UDPglc}) \times [\text{Ra}(\text{glc};\text{endo}) + \text{Inf}(\text{glc};\text{total})]$
17. glc(UDPglc)	$c(\text{glc}) \times [\text{Ra}(\text{UDPglc};\text{endo}) + \text{Inf}(\text{gal};\text{total})]$
18. GNG(glc)	$f(\text{glc}) \times [\text{Ra}(\text{glc};\text{whole body}) + \text{Rr}(\text{glc})]$
19. GNG(UDPglc)	$f(\text{UDPglc}) \times [\text{Ra}(\text{UDPglc};\text{whole body}) + \text{Rr}(\text{UDPglc})]$
20. GNG(glc;indirect)	$[f(\text{UDPglc}) \times \text{UDPglc}(\text{glc})] + [f(\text{glc}) \times \text{Rr}(\text{glc})]$
21. GNG(UDPglc;indirect)	$[f(\text{glc}) \times \text{glc}(\text{UDPglc})] + [f(\text{UDPglc}) \times \text{Rr}(\text{UDPglc})]$
22. GNG(glc;direct)	$\text{GNG}(\text{glc}) - \text{GNG}(\text{glc};\text{indirect})$
23. GNG(UDPglc;direct)	$\text{GNG}(\text{UDPglc}) - \text{GNG}(\text{UDPglc};\text{indirect})$
24. GLY(glc)	$\text{Ra}(\text{glc};\text{endo}) - \text{GNG}(\text{glc};\text{direct}) - [f(\text{UDPglc}) \times \text{UDPglc}(\text{glc})]$
25. GLY(UDPglc)	$\text{Ra}(\text{UDPglc};\text{endo}) - \text{GNG}(\text{UDPglc};\text{direct}) - \text{glc}(\text{UDPglc})$
<i>Individual fluxes comprising hepatic G6P metabolism</i>	
26. GNG(G6P)	$\text{GNG}(\text{glc};\text{direct}) + \text{GNG}(\text{UDPglc};\text{direct})$
27. GK	$\text{glc}(\text{UDPglc}) + \text{Rr}(\text{glc})$
28. G6Pase	$\text{GNG}(\text{glc}) + \text{GLY}(\text{glc})$
29. GS	$\text{GNG}(\text{UDPglc}) + \text{GLY}(\text{UDPglc})$
30. GP	$\text{GLY}(\text{UDPglc}) + \text{GLY}(\text{glc}) + \{[1 - c(\text{glc})] \times \text{Rr}(\text{UDPglc})\}$



*Legend Supplementary Figure 1*

Schematic model of hepatic carbohydrate metabolism. Major metabolic pathways and enzymatic reactions are depicted, despite glycolysis, sharing glucose-6-phosphate (G6P) as a central metabolite. The pathways included are: (1) Gluconeogenic flux toward G6P, (2) Glycogen phosphorylase flux, (3) Glucose-6-phosphatase flux, (4) Glucokinase flux and (5) Glycogen synthase flux. Mice received an infusion containing [U-<sup>13</sup>C]glucose, [2-<sup>13</sup>C]glycerol, [1-<sup>2</sup>H]galactose and paracetamol for six hours. Mass isotopomer distribution analysis (MIDA) was applied on blood glucose and urinary paracetamol glucuronide samples.

# Supplementary Figure 1



***Lxr $\alpha$*  Deficiency Hampers the Hepatic Adaptive Response to Fasting in Mice**

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